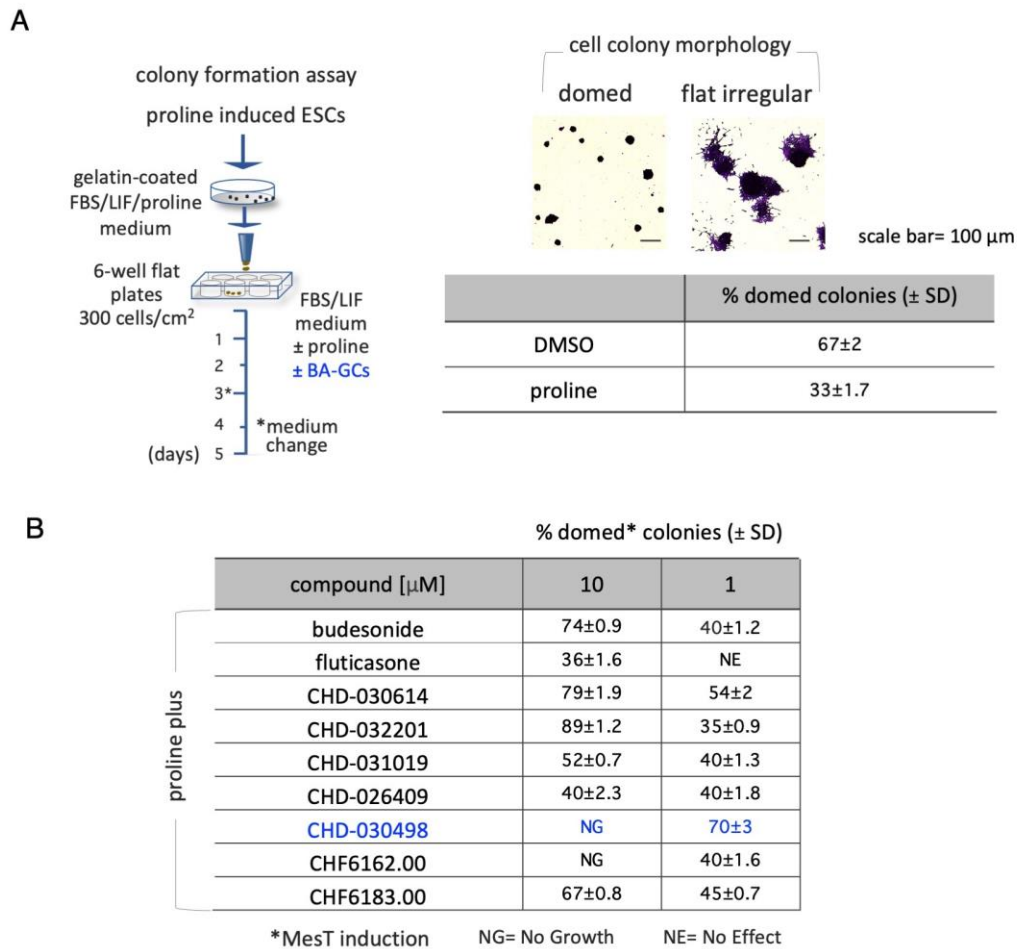
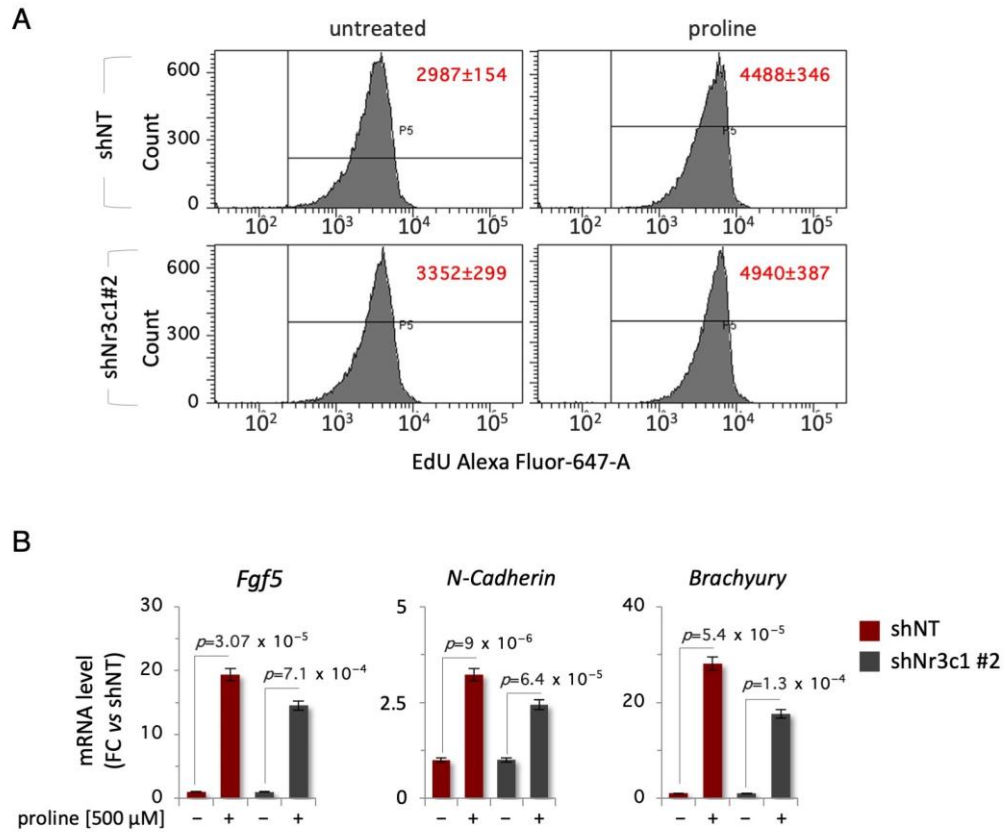


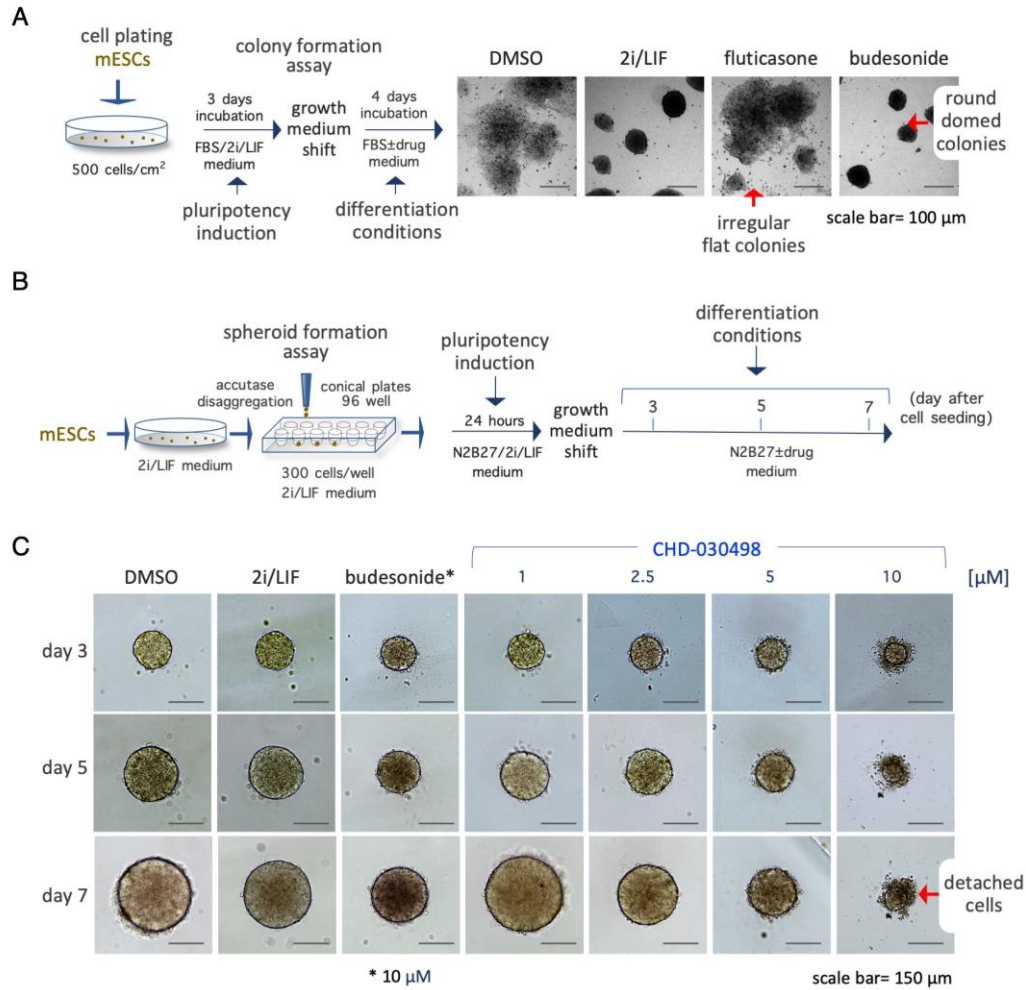
Supplementary Figure S1. Effect of BA-GCs on ESCs proliferation. (A) Effect of BA-GCs on ESC survival at the following concentrations: 10^{-10} , 10^{-9} , 10^{-8} , 10^{-7} , 10^{-6} , 10^{-5} M, for 48 hrs, by CCK-8 assay. Data are shown as mean \pm SD ($n = 3$; $p \leq 0.01$). (B) FACS analysis of apoptotic cells. Representative dot plot of Annexin V/PI staining in ESCs treated with DMSO as Control, and treated with the different BA-GCs used at the indicated concentrations. The fraction (%) of Annexin V+ (bottom right) and Annexin V+ PI+ (top right) cells are shown. Annexin V is conjugated with FITC. (C) FACS analysis of EdU incorporation. Representative dot plot of EdU incorporation in ESCs treated with DMSO as Control, and treated with BA-GCs at the indicated concentrations. The fraction (%) of cells that incorporated EdU is shown.



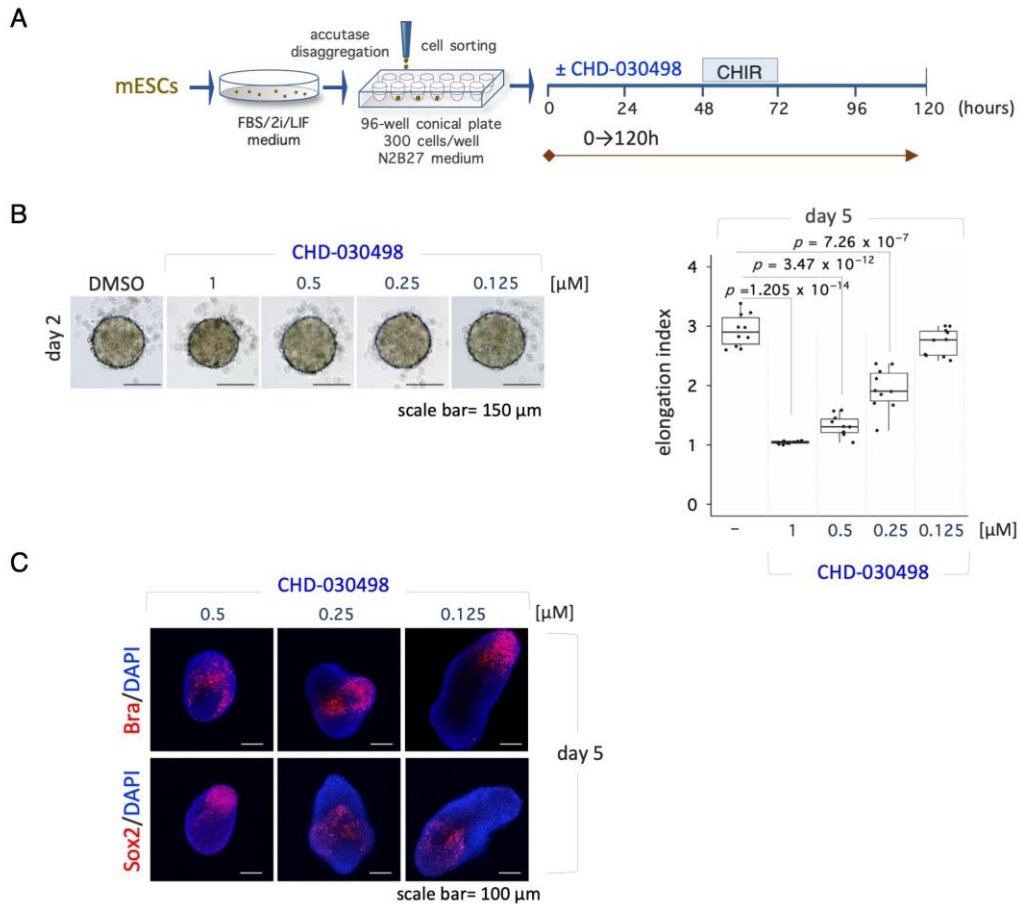
Supplementary Figure S2. Effect of BA-GCs on mesenchymal-to-embryonic stem cell transition (MesT). (A) Schematic representation of the experimental procedure (left). Representative photomicrographs of domed- and flat-shaped cell colonies (top right) and quantification of the fraction (%) of domed-shaped cell colonies generated from proline-induced mesenchymal-like cells treated ± proline (250 μM) (bottom right). (B) Table showing the fraction (%) of domed-shaped cell colonies (MesT induction), generated from proline-induced mesenchymal-like cells treated with proline (250 μM) ± BA-GCs at the indicated concentrations. Budesonide and fluticasone were used as a positive (MesT inducer) and negative (inactive) Control. Data are shown as mean ± SD (n=3).



Supplementary Figure S3. Effects of proline on the proliferation of GR knock-down cells. (A) FACS analysis of proliferating cells. Representative histograms of EdU incorporation in shNT and shNr3c1#2 ESCs \pm proline (500 μ M) are shown. The mean intensity of EdU incorporation is shown. Data are mean \pm SEM (n=3). (B) Effect of GR on the expression of esMT marker genes. qPCR analysis of Fibroblast Growth Factor 5 (*Fgf5*), Neural Cadherin (*N-Cadherin*) and Brachyury gene expression. Values are expressed as a fold-change *versus* shNT ESCs, after normalization to *Gapdh* and are mean \pm SEM (n=3).



Supplementary Figure S4. Time-course and dose-response effect of CHD-030498 on pluripotency exit. (A) Schematic representation of the pluripotency exit procedure (left). ESCs were plated (500 cells/cm²) in DMEM/FBS/2i/LIF medium for 3 days, and then shifted to DMEM/FBS medium supplemented with DMSO, 2i/LIF, fluticasone, or budesonide. After 4 days of incubation the cell colonies were imaged (bright-field images; right). Scale bar, 100 µm. (B) Schematic representation of the experimental procedure. ESCs (2i/LIF) were FACS sorted (300 cells/well) on 96-well ultra-low conical plates and incubated in N2B27 ± DMSO, 2i+LIF or CHD-030498 (from 1 to 10 µM). (C) Representative bright-field images of spheroids obtained as described in (B). Images were captured for each condition at day 3, 5 and 7, and measured. Scale bar, 150 µm.



Supplementary Figure S5. Dose-response activity of CHD-030498 on gastruloid development. (A) Schematic representation of the experimental design. Naïve pluripotent (2i/LIF) ESCs were FACS sorted (300 cells/well) on 96-well ultra-low conical plates in N2B27 \pm CHD-030498 added at 1, 0.5, 0.25 and 0.125 μ M, from t0 to t120 hrs. (B) Representative bright-field images (left) and boxplot diagrams of gastruloid elongation index (right) of 2-day-old aggregates treated with DMSO (control), or CHD-030498 at 1, 0.5, 0.25 and 0.125 μ M ($n = 3$; 20 gastruloids/condition). Scale bar, 150 μ m. (C) Immunofluorescence analysis of differentiation (Brachyury, Sox2) markers. Representative confocal images of 5-day-old aggregates/gastruloids treated with CHD-030498 added at the indicated concentrations. Nuclei were counterstained with DAPI (blue). Scale bar, 100 μ m.